REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From – To)*
07-09-2005	Final Report	23 Jun 2003 to 22 Mar 2005

4. TITLE AND SUBTITLE

The relationship between copper speciation and toxicity for larvae of the blue mussel *Mytilus edulis*: Regulatory Implications and determination of water effect ratios.

5a. CONTRACT NUMBERS

5b. GRANT NUMBER

N00014-03-1-0918

5c. PROGRAM ELEMENT NUMBER

ONR 331

6. AUTHOR(S)
James W. Moffett

5d. PROJECT NUMBER

03PR13195-00

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Woods Hole Oceanographic Institution
Woods Hole MA 02540

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Office of Naval Research
Ballston Tower 1
800 N Quincy Street
Arlington VA 22217-5660

10. SPONSORING/MONITORING ACRONYM(S)

11. SPONSORING/MONITORING AGENCY REPORT NUMBER

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Copper toxicity to larvae of the blue mussel Mytilus edulis was quantified in chemically defined media to determine the relationship between concentrations of the free aquo Cu^{2+} ion and toxicity. The objective was to generate robust relationships to predict the effects of water chemistry on the toxic effects of copper and assess their ecological and regulatory implications.
We found a robust relationship exists, suggesting that this species, a key organism in the regulatory enforcement community, is affected primarily by free Cu ions. This validates current assumptions behind the adoption of site-specific criteria derived from testing. However, the concentrations of free Cu ions required to observe chronic toxic effects (10-9M) are much higher than found in most harbors. Titration data in the literature suggest that dissolved concentrations of Cu in US harbors would have to be much higher before the survival of this organism was threatened. This is significant because an exhaustive study of Cu toxicity to invertebrates carried out in the 1970s found that Mytilus larvae were among the most sensitive species. It seems likely that concerns about Cu toxicity to marine planktonic invertebrates may be overstated. Caution must be exercised when extending these conclusions to benthic organisms, to which more refractory forms of Cu are probably bioavailable.

15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON James W Moffett
a. REPORT	b. ABSTRACT	c. THIS PAGE			19 b. TELEPHONE NUMBER *(Include are code)* (508) 289 3218

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI-Std. Z39-18

Final Report

The blue mussel *Mytilus edulis*, is an ecologically and economically important species in coastal US waters. It is also the primary species used to establish water quality criteria for copper by the EPA and other regulatory agencies. These criteria are used to determine NPDES permit levels for Cu that have important financial and logistical implications for US Naval bases and shipyards.

The larvae of *Mytilus edulis* were singled out as particularly vulnerable in an exhaustive survey carried out in the 1970s, and criteria were established based on toxicity tests in waters from Narragansett Bay. Subsequently, it was shown that waters with more dissolved organic matter require more Cu to be added to achieve the same toxic effect. This is hypothesized to be due to strong complexation by organic ligands which lower Cu bioavailability. This interpretation has provided a rationale for site specific criteria to be developed in harbors with high organic matter concentrations.

The theoretical basis for this change is the free ion model of toxicity, which suggests that toxicity is proportional to free Cu^{2+}, rather than total Cu. This model has been used for free Cu^{2+} and shown to hold for many unicellular phytoplankton using natural and artificial ligands, but has never been evaluated for *Mytilus*. Indeed, nobody has ever shown that the amelioration of Cu toxicity to *Mytilus* in high organic waters is actually due to complexation.

In this study, we quantified the relationship between *Mytilus* toxicity and free Cu^{2+} in EDTA buffered seawater media. We also looked at toxicity in waters containing high (coastal) and low (blue water) concentrations of dissolved organic matter.

We collaborated with Pacific Eco-Risk, a consulting company with a long track record in performing *Mytilus* tests with other researchers from organizations such as HydroQual. Results indicated that there was a robust relationship between free Cu^{2+} and toxicity in both the EDTA buffered samples and in the natural samples, with chronic toxicity around a free Cu^{2+} concentration of 10^{-9} M.

These results are important for several reasons.

1. **Predictive Capabilities** They allow us to assess the toxicity of Cu to *Mytilus* in estuaries around the world based on the large data set in the literature. Moreover, because most of these data are titrations, we can assess how increasing total Cu in the future might affect *Mytilus*.

2. **Environmental Implications** There are few, if any coastal waters where the free Cu^{2+} is as high as 10^{-9} M. This is because total dissolved Cu rarely exceeds 10^{-7} M (about 6ppb) even in contaminated waters, and even at those levels there are usually enough ligands to bind 90% of the Cu. Therefore, our results suggest that Mytilus larvae are not threatened by Cu to a significant extent anywhere in the coastal US. However, further increases in Cu (by a factor of 2 or 3) may well pose a significant threat.

3. **Validation of site specific Criteria Approach** The robust relationship between free Cu^{2+} and toxicity observed in the real samples suggests that differences in *Mytilus* toxicity thresholds among different waters do reflect organic complexation and thus can

be generalized to other organisms where toxicity is controlled by the free ion model. Our work therefore constitutes a molecular-level validation of the site-specific criteria/water effect ratios approach.

Current Status

Currently we are synthesizing our data for publication in Environmental Science and Technology.